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13. ABSTRACT (Maximum 200 Words)

Despite advances in the detection and treatment of breast cancer, the final impact on survival rates has not altered significantly. Based on these considerations, novel therapeutic approaches, such as gene-based therapy, offer promise for successfully treating this prevalent female disease. I am constructing replication competent bipartite adenoviruses (RCBA) in which the replication is controlled by the promoter of the PEG-3 gene (PEG-Prom) that shows cancer-selective activity and which simultaneously express either the cancer-selective apoptosis-inducing gene melanoma differentiation associated gene-7 (mda-7)/IL-24 or an immunomodulatory gene such as interferon-γ or IL-2. I have demonstrated that PEA-3 and AP-1, transcription factors involved in regulating oncogenesis and tumor progression, are involved in the regulation of PEG-prom activity in breast cancer cells. I have also demonstrated that RCBA in which replication is controlled by PEG-Prom and which expresses mda-7/IL-24 (Ad.PEG-E1A.CMV-mda-7) kills breast cancer cells without harming normal breast epithelial cells. However, RCBA in which replication is controlled by CMV promoter (Ad.CMV-E1A.CMV-mda-7) kills both breast cancer and normal cells indicating the breast cancer-selective killing property of Ad.PEG-E1A.CMV-mda-7. This exciting initial observation indicates that Ad.PEG-E1A.CMV-mda-7 might be a valuable tool for actual therapeutic application in patients with breast cancer.

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Introduction

Despite advances in the detection and treatment of breast cancer, the final impact on survival rates has not altered significantly. Currently, one in nine American women can be expected to develop breast cancer, of which one third will die of this disease (1). A high priority exists for developing approaches to improve the long-term disease-free status and overall survival of patients. The objective of the proposal is to develop effective adenovirus-based gene therapy approach for the treatment of breast cancer. This approach contains three basic elements. (**1**) It utilizes PEG-Prom, the promoter region of the rodent Progression elevated gene-3 (PEG-3), which has selectively high activity in cancer cells, including breast cancer cells, while having very low to no activity in normal cells, including primary breast epithelial cells (2, 3 and unpublished data). (**2**) The PEG-Prom is used to drive the expression of E1A and E1B genes of adenovirus, which are necessary for its replication. This strategy allows conditional replication of adenovirus only in cancer cells but not in normal cells. (**3**) These conditionally replicating adenoviruses express pro-apoptotic or immuno-modulatory genes such melanoma differentiation associated gene-7 (mda-7)/IL-24 or interferon-γ or IL-2 thus allowing profound anti-tumor apoptotic and immune response. Importantly, multiple studies have shown that mda-7/IL-24 has cancer-selective apoptosis-inducing properties without having any adverse effect on normal cells (4-8). Thus the combination of PEG-Prom and mda-7/IL-24 will provide cancer-specific expression of transgene and cancer-specific killing. The specific aims of the three-year proposal are (**1**) To molecularly characterize the PEG-Prom to understand how it functions and to more effectively use it to target human breast cancers. (**2**) To construct replication competent bipartite adenovirus (RCBA) vectors in which the PEG-Prom controls expression of E1A and a cytomegalovirus (CMV) promoter controls expression of *mda*-7/IL-24 or an immunomodulatory gene (IFN-γ or IL-2). (**3**) To evaluate the ability of RCBAs to inhibit *in vivo* growth of established human breast cancer xenografts in athymic nude mice. Specific aim 1 would be accomplished in Year 1, specific aim 2 in Year 2 and specific aim 3 in Year 3. However, because of the complex technical methods of making RCBAs, I have started to perform experiments that encompass both Specific aims 1 and 2 and have partially accomplished both these aims. I am continuing my experiments so that both the aims are finished by Year 2.

Body

During the past year I have obtained significant data with regards to specific aims 1 and 2 which are enumerated below.

I. PEA-3 and AP-1 are important for regulation of PEG-Prom activity in breast cancer cells. In rodent system, it was observed that PEA-3 and AP-1 transcription factors play important roles in controlling the activity of PEG-Prom (2, 3). I, therefore, checked the involvement of these two factors in regulation of PEG-Prom activity in breast cancer cells. This is especially important since PEA-3 has been shown to be overexpressed in breast carcinoma cells (9, 10). Mutant constructs were made in which either PEA-3 or AP-1 or both sites were mutated and the luciferase activity of these constructs were analyzed in a number of breast cancer cells. In all the cell lines, mutation of either PEA-3 or AP-1 site decreased the promoter activity by ~50%, while mutation of both sites reduced it to ~25% (**Fig. 1**). The basal promoter activity was much higher in MCF-7 and T47D cells than in MDA-MB-157 and MDA-MB-231 cells. I am currently examining if there is any correlation between the promoter activity and the expression levels of PEA-3 and AP-1 in each cell line.

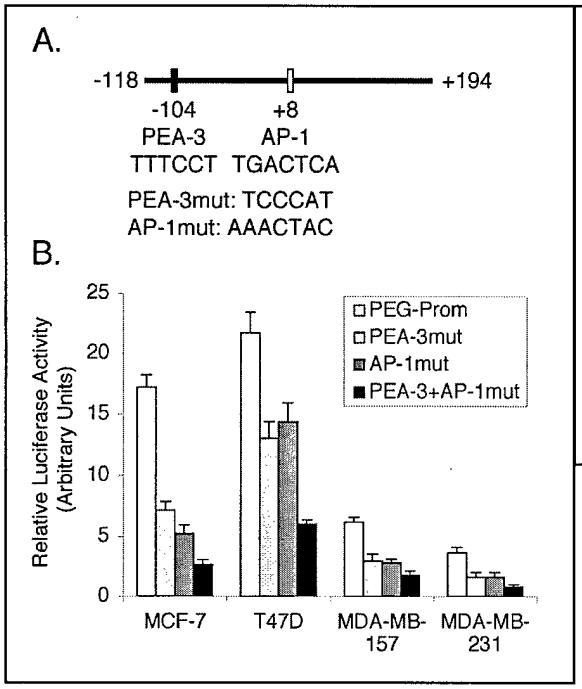

Fig. 1. PEA-3 and AP-1 are involved in the regulaiotn of PEG-Prom activity. **A.** Schematic representation of PEG-Prom showing the position of PEA-3 and AP-1 sites. The numbers represent the relative positions of the nucleotide when the transcription start site is regarded as +1. **B.** The indicated cell lines were transfected with the different promoter-luciferase constructs together with a β-galactosidase expression construct. Luciferase assay was performed 48 h post-transfection and luciferase expression was normalized by β-galactosidase expression. The data represent mean±S.D. of two independent experiments

II. Construction of Ad.PEG-E1A.CMV-mda-7 and Ad.CMV-E1A.CMV-mda-7. I constructed Ad.PEG-E1A.CMV-mda-7 in which PEG-Prom drives the expression of adenoviral E1A gene and CMV promoter controls the expression mda-7 gene in the E3 region of the adenovirus. As a control I constructed Ad.CMV-E1A.CMV-mda-7 in which

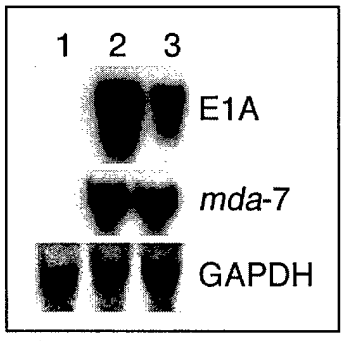

Fig. 2. RCBAs generate E1A and mda-7 mRNAs. T47D breast cancer cells were infected with Ad.vec (lane 1), Ad.CMV-E1A.CMV-mda-7 (lane 2) and Ad.PEG-E1A.CMV-mda-7 (lane 3) at an M.O.I. of 100 pfu/cell. The expressions of E1A, mda-7 and GAPDH were analyzed by Northern blot analysis 24 h post-infection

CMV promoter controls the expression of both E1A and mda-7 genes. Two additional constructs were created as controls, Ad.CMV-E1A and Ad.PEG-E1A in which the expression of E1A was controlled by CMV promoter and PEG-Prom, respectively,

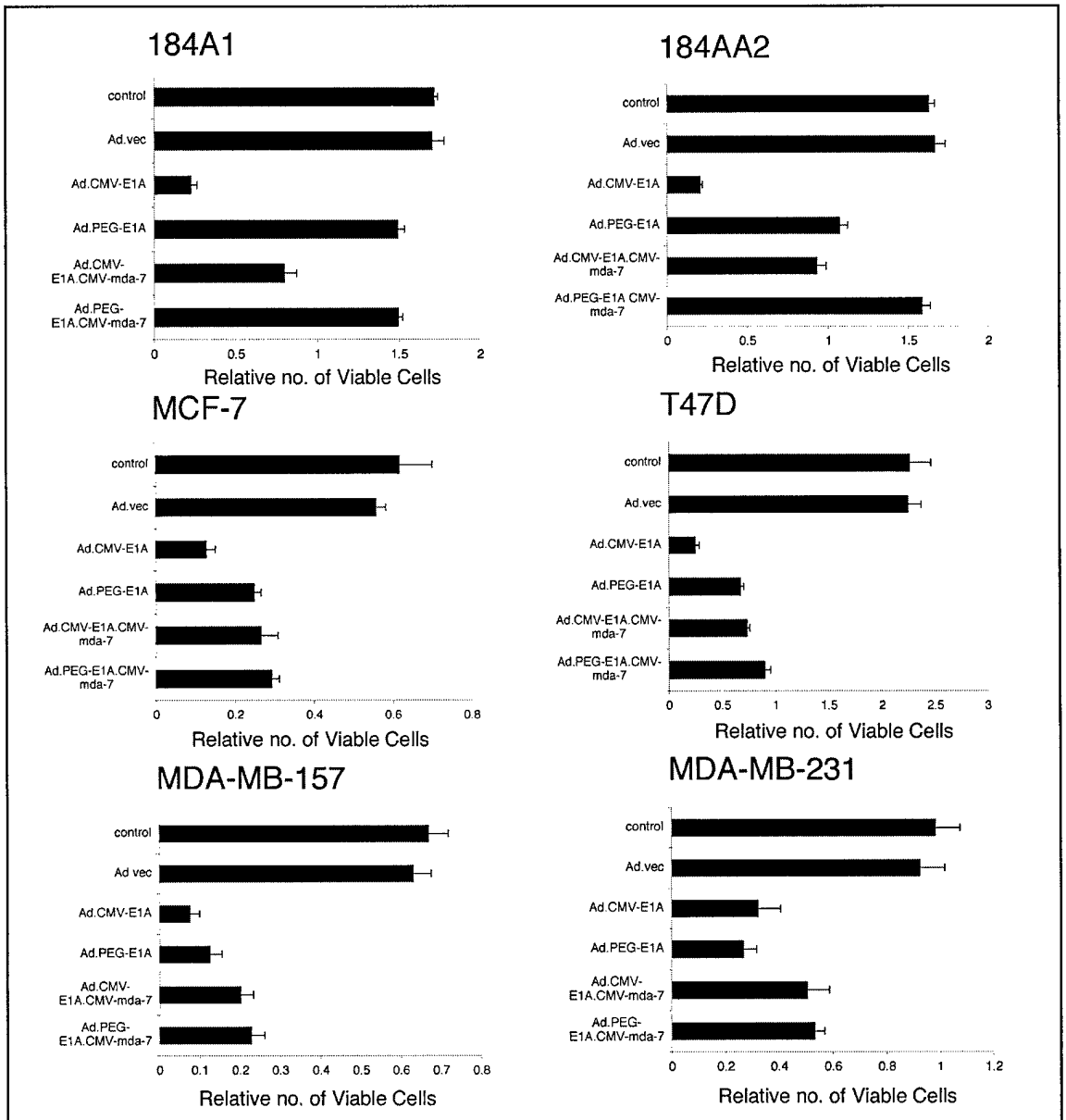

Fig. 3. Breast cancer specific killing of PEG-Prom-driven adenoviruses. Different cell lines were infected with the indicated adenoviruses at an M.O.I. of 100 pfu/cell and cell viability was analyzed by standard MTT assay 4 days post-infection. The data represent mean±S.D. of two independent experiments.

leaving the E3 region empty. Both Ad.CMV-E1A.CMV-mda-7 and Ad.PEG-E1A.CMV-mda-7 successfully generated E1A and mda-7 mRNAs (**Fig. 2**) indicating the authenticity of these constructs.

III. Breast cancer specific killing of PEG-prom-driven adenoviruses. I infected 184A1 and 184AA2, two immortal but non-tumorigenic breast epithelial cells, and MCF-

7, T47D, MDA-MB-157 and MDA-MB-231 breast cancer cells with Ad.vec (control empty adenovirus), Ad.CMV-E1A, Ad.PEG-E1A, Ad.CMV-E1A.CMV-mda-7 and Ad.PEG-E1A.CMV-mda-7 and analyzed cell viability by standard MTT assay. Ad.PEG-E1A and Ad.PEG-E1A.CMV-mda-7 showed little to no adverse effect on the viablitiy of 184A1 and 184AA2 cells since PEG-Prom does not function in normal cells and since mda-7 induces apoptosis only in cancer cells but not in normal cells (**Fig. 3**). However, Ad.CMV-E1A and Ad.CMV-E1A.CMV-mda-7 could inhibit the growth of these cells indicating the specificity of action of PEG-Prom in cancer cells. All the adenoviruses could significantly inhibit the growth of all the breast cancer cells. These findings indicate that Ad.PEG-E1A.CMV-mda-7 might be developed as an effective tool for eradication of breast cancer. I am currently making other RCBAs which I will characterize in the year to come.

Key Research Accomplishments:

1. Identification of PEA-3 and AP-1 transcription factors as key elements regulating the activity of PEG-Prom in breast cancer cells.
2. Construction of RCBAs, Ad.PEG-E1A.CMV-mda-7 and Ad.CMV-E1A.CMV-mda-7.
3. Demonstration of breast cancer-specific killing of Ad.PEG-E1A.CMV-mda-7 without having adverse effect on normal breast epithelial cells.

Reportable Outcomes:

Publications:

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cell lines through JAK/STAT-independent pathways. J Cell Physiol. 2003, 196: 334-345.

13. Yacoub A, Mitchell C, Lister A, Lebedeva IV, Sarkar D, Su Z-Z, Sigmon C, McKinstry R, Ramakrishnan V, Qiao L, Broaddus WC, Gopalkrishnan R, Grant S, Fisher PB, Dent P. Melanoma differentiation-associated 7 (interleukin 24) inhibits growth and enhances radiosensitivity of glioma cells *in vitro* and *in vivo*. Clin Cancer Res. 2003, 9:3272-3281.
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Presentations at Scientific Meetings

1. Sarkar D, Su Z-Z, Lebedeva IV, Fisher PB. Exploiting defects in molecular circuitry as a novel approach for the therapy of pancreatic cancer. LustGarten Foundation, June, 2004.
2. Sarkar D, Leszczyniecka M, Kang D-C, Valerie K, Pandita TK, Fisher, PB. Induction of senescence-like growth arrest in melanocytes and malignant melanoma cells by human polynucleotide phosphorylase (*hPNPase* $^{old-35}$). 95th Annual Meeting of American Association for Cancer Research, Proceedings, #2413, Orlando, March, 2004.
3. Lebedeva IV, Su Z-Z, Sarkar D, Gopalkrishnan RV, Waxman S, Yacoub A, Dent P, Fisher PB. Strategy for promoting apoptosis selectively in pancreatic cancer cells. 95th Annual Meeting of American Association for Cancer Research, Proceedings, #2945, Orlando, March, 2004.
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Conclusions:

I have demonstrated that RCBA in which the replication is regulated by cancer-selective PEG-Prom and that expresses cancer-selective apoptosis inducing cytokine mda-7/IL-24 (Ad.PEG-E1A.CMV-mda-7) specifically kills breast cancer cells without harming normal breast epithelial cells. However, RCBA in which replication is controlled by CMV promoter (Ad.CMV-E1A.CMV-mda-7) kills both breast cancer and normal cells. These preliminary findings indicate that Ad.PEG-E1A.CMV-mda-7 might be developed as a valuable reagent for gene therapy of breast cancer. The efficacy of the RCBA will be tested in eradicating breast cancer xenografts in nude mice that will pave the way for translating this RCBA to actual therapeutical application in breast cancer patients.

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